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Parallel reaction monitoring MS

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An abbreviated version of this protocol was published in Science Advances in Dec 2020

Metabolomic shifts associated with heat stress in coral holobionts

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Detailed protocol

Parallel reaction monitoring mass spectrometry. Metabolic features having correlative trends to heat stress were first identified from the full-scan MS runs. These metabolic features were then subjected to parallel reaction monitoring (PRM) to generate high quality MS^2 spectra. We also collected the high quality MS^2 spectra from synthetic standards in order to confirm the metabolic features identified from the coral samples. For each dipeptide verified in the manuscript, multiple isomer matches existed based on the exact mass and key feature aspects as determined by the MS^2 spectra (Table below). The specific instrument settings of each PRM method event are include in the table below.

Coral Dipeptide	Peptide Standard	RT (min)	RT (max)	m/z (Neg)	m/z (Pos)	(N)CE	Spectrum Type
$C_{11}H_{22}N_6O_4$ (RT 9.05min)	**RQ**	**0**	**22**	**301.16298**	**303.17753**	**30,55,80**	**Centroid**
	QR	0	22	301.16298	303.17753	30,55,80	Centroid
	ER-NH2	0	22	301.16298	303.17753	30,55,80	Centroid
	RE-NH2	0	22	301.16298	303.17753	30,55,80	Centroid
	γ-ER-NH2	0	22	301.16298	303.17753	30,55,80	Centroid
	AGR	0	22	301.16298	303.17753	30,55,80	Centroid
	RAG	0	22	301.16298	303.17753	30,55,80	Centroid
	RGA	0	22	301.16298	303.17753	30,55,80	Centroid
$C_{11}H_{22}N_4O_4$ (RT 9.96min)	**KQ**	**0**	**22**	**273.15683**	**275.17138**	**30,55,80**	**Centroid**
	QK	0	22	273.15683	275.17138	30,55,80	Centroid
	EK-NH2	0	22	273.15683	275.17138	30,55,80	Centroid
	KE-NH2	0	22	273.15683	275.17138	30,55,80	Centroid
	γ-EK-NH2	0	22	273.15683	275.17138	30,55,80	Centroid
$C_{11}H_{23}N_5O_3$ (RT 7.64min)	**RV**	**0**	**22**	**272.17281**	**274.18737**	**30,55,80**	**Centroid**
	VR	0	22	272.17281	274.18737	30,55,80	Centroid
$C_9H_{19}N_5O_3$ (RT 8.97min)	**RA**	**0**	**22**	**244.14151**	**246.15607**	**30,55,80**	**Centroid**
	AR	0	22	244.14151	246.15607	30,55,80	Centroid

The MS^2 spectra generation was performed on a Thermo Q Exactive PLUS with a HESI source which was set to a spray voltage of -2.7kV under negative mode and 3.5kV under positive mode. The sheath, auxiliary, and sweep gas flow rates of 40, 10, and 2 (arbitrary unit) respectively. The capillary temperature was set to 300°C and aux gas heater was 360°C. The S-lens RF level was 45. The AGC target was set to 2e5 and the maximum IT was 100ms. The resolution was set to 70,000. The isolation window was set to 2.0 m/z. Collision energy levels were set to 30, 55 and 80 NCE.

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1. Chiles, E. , Williams, A. , Bhattacharya, D. and Su, X. (2021). Parallel reaction monitoring MS. Bio-protocol Preprint. bio-protocol.org/prep1138.
2. Williams, A., Chiles, E. N., Conetta, D., Pathmanathan, J. S., Cleves, P. A., Putnam, H. M., Su, X. and Bhattacharya, D.(2020). Metabolomic shifts

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